University explained at the symposium, they open up some intriguing new vistas on quantum gravity itself. For example:

■ Ashtekar's variables combine information about the curvature of space with information about the evolution of curvature in time. As a result, they transform Einstein's highly nonlinear equations into much more tractable quadratic equations. Indeed, the dynamics of gravity comes to resemble the dynamics of a simple harmonic oscillator—a system whose solution can be found in any elementary quantum mechanics text.

■ Ashtekar's approach depends heavily on the use of spinors, which are the natural mathematical structures for describing relativistic particles such as quarks, electrons, and neutrinos. Indeed, Einstein's equations in their new form are closely analogous to the so-called Yang-Mills equations, which are used in conventional particle physics to formulate the grand unified theories of the strong, weak, and electromagnetic interactions. The new variables may thus point the way toward physicists' Holy Grail: an even grander unification that incorporates all four forces.

■ In Ashtekar's formulation the quantum gravity equations can be solved, at least partially. The intriguing thing, as Smolin has shown in collaboration with Ted Jacobson of Brandeis University, is that the solutions can best be expressed in terms of integrals of the new variable around closed paths in space. Moreover, the dynamics of quantum gravity seems to involve the splitting and joining of these paths wherever they cross each other. The whole thing is eerily reminiscent of the splitting and joining that goes on in the currently fashionable theory of superstrings. Since superstrings are also supposed to describe quantum gravity (although not precisely Einstein's gravity), Ashtekar's variables may be pointing to a deep connection.

■ Finally, said Smolin, the fundamental geometric object in Einstein's theory—the so-called metric tensor—is an inherently quantum object in Ashtekar's formulation. Like position and momentum in ordinary quantum mechanics, it is subject to the uncertainty principle, and it can only be defined in terms of probabilities and averages. This means that, on the very smallest scales, the structure of space and time is subject to violent fluctuations. Indeed, said Smolin, on the smallest scales space and time may not *have* any well-defined structure. They may be more like a kind of "quantum foam." This idea has been around for many years, he added. But Ashtekar's formulation of quantum gravity may be yielding the first, mathematically precise definition of it. ■

M. MITCHELL WALDROP

A New Wave of Enzymes for Cleaving Prohormones

Researchers have isolated several enzymes that snip peptide hormones from the inactive prohormones. Some may not stand the test of time

All peptide hormones and neurotransmitters are synthesized in large inactive "prohormones" that must be cleaved at specific sites to release the active agents. Although researchers have searched for many years for the enzymes that perform the cleavages, they have had little luck until recently. They have now produced what one investigator calls "an embarrassment of riches." Several proteases that appear to have the required specificities have been isolated and are vying for consideration as cleaving enzymes.

Most of these proteases are clearly different from one another, a diversity that may simply reflect the existence of distinct cleaving enzymes for different prohormones. Alternatively—and perhaps more likely—some of the isolated enzymes may be red herrings that do not actually participate in peptide hormone synthesis. Proving that an isolated protease is physiologically active in cleaving peptide hormones from prohormones, and not just one of the many cellular degradative enzymes, is very difficult.

The pattern in which physiologically active peptides are synthesized within larger prohormones has been highly conserved in evolution and is found in organisms as simple as yeast and as complex as man. In fact, the protease with the clearest claim to being a prohormone-cleaving enzyme was identified about 2 years ago in *kex2*, a yeast mutant, by Jeremy Thorner, David Julius, and their colleagues at the University of California in Berkeley. This enzyme cuts the yeast α-factor, a peptide that contains 13 amino acids and is necessary for sexual mating in yeast, from its precursor protein.

A great deal of evidence indicates that prohormones are converted to the active hormones in cells within the small membrane-enclosed vesicles called secretory vesicles. Because the cells of mammals and other higher organisms are not amenable to the same types of genetic analysis and manipulation that led to the discovery of the *kex2* enzyme, many investigators therefore try to improve their odds of coming up with physiologically important cleaving enzymes by purifying the secretory vesicles first and using them as a starting material for isolating the target enzyme. For example, Y. Peng Loh and her colleagues at the National Institute of Child Health and Human Development took this approach to look for enzymes that cleave pro-opiomelanocortin (POMC), a prohormone that is made in the pituitary gland and contains within its structure no fewer than seven active peptides.

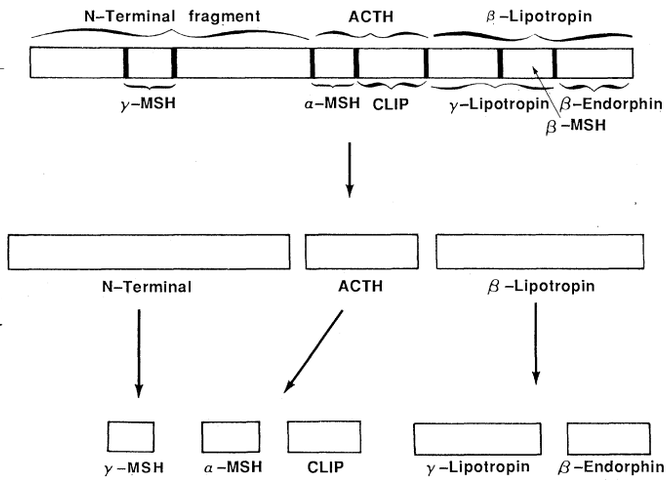
The Loh group has isolated from the pituitary gland two POMC-cleaving enzymes that are at least very similar to one another and may be identical. According to Loh, the enzymes cut POMC at the same three sites at which it is initially cleaved in the pituitary, which means that the proteases satisfy one of the criteria that must be met by authentic cleavage enzymes. Recent work indicates that the enzymes also accurately cleave proinsulin to insulin and provasopressin to vasopressin, findings that suggest that the enzymes may have a broader spectrum of activity in the body than was originally thought.

Several proteases that appear to have the required specificities have been isolated . . .

In the intermediate lobe of the pituitary the products released by the initial set of three cuts are further split. Loh's enzyme is not capable of this second round of cleavages, however. This finding suggests that at least one additional enzyme is needed to convert POMC to its final products in the intermediate lobe.

A second criterion that must be met by a prohormone-cleaving enzyme is that it must be active at the *p*H of the secretory vesicles which is generally taken to be somewhat acidic, around 5 to 6. The *p*H optimum of the POMC-cleaving enzyme is in the range of 4.0 to 5.0, Loh says, and it should therefore work in the vesicles. In addition compounds that inhibit the activity of the

Cleavage of pro-opiomelanocortin.



The active peptides released from this prohormone include the lipotropins, adrenocorticotropic hormone (ACTH), β-endorphin, three forms of melanocyte-stimulating hormone (α-, β-, and γ-MSH), and corticotropin-like intermediate lobe peptide (CLIP). The dark bars in the POMC molecule indicate the pairs of basic amino acids that mark the ends of the active peptides and are the targets for the cleaving enzyme or enzymes. Such basic amino acid pairs generally delineate the active peptides within prohormones.

enzyme also depress POMC cleavage in pituitary cells. "As far as anyone has put up criteria [for prohormone-cleaving enzymes], this enzyme has met them," Loh concludes.

Nevertheless, Michel Chretien, Nabil Seidah, and their colleagues at the Clinical Research Institute of Montreal have isolated a different pituitary protease. They are equally convinced that they have identified a cleaving enzyme for POMC and other prohormones. "We believe at this stage that this is a key enzyme in the maturation of these peptides," Chretien asserts.

The Montreal workers have shown that the protease produces physiological cleavages of the POMC derivatives adrenocorticotropic hormone, β-lipotropin, and the amino-terminal glycoprotein, although they have not tested its activity with POMC itself. This enzyme also appears to have a broad spectrum of activity. In addition to cleaving the POMC derivatives, it cuts proinsulin, another prohormone containing the endogenous opioids known as enkephalins, and also the prohormone for atrial natriuretic factor, a heart peptide that helps to regulate the blood pressure. Chretien and Seidah have identified a protease in the heart that may be identical to the brain enzyme, a finding consistent with their hypothesis that the enzyme plays a key role in the maturation of several prohormones.

The pituitary enzyme isolated by the Montreal group, which proved to belong to a catalytic class of proteases different from that of the Loh enzyme, has a *p*H optimum of 8. According to Seidah, however, the high *p*H optimum of the serine protease does not disqualify it from consideration as a prohormone cleavage enzyme. He points out that the *p*H of newly formed secretory vesicles may be higher, around 7 or so, than in the mature vesicles, to which the *p*H range of 5 to 6 applies. "We think that the acidic *p*H is there to kill the enzyme, not to let it work," Seidah says. Continued activity of a cleaving enzyme after it has done its job might be deleterious if it destroys active hormones before they can be released.

At present, it is not possible to tell whether the protease of the Loh group and that of the Chretien-Seidah group will both prove to be physiologically important as broad-spectrum cleavage enzymes or whether one—or both—will eventually fall by the wayside. Moreover, the situation is further complicated by the existence of additional proteases that have been isolated from mammals and other animals and cleave some of the same prohormones acted upon by the proteases of Loh and Chretien and Seidah.

The other candidates include an enzyme that has been implicated in proinsulin cleavage by Donald Steiner and his colleagues at the Howard Hughes Medical Institute Research Laboratories and the University of Chicago School of Medicine. This protease resembles cathepsin B, one of the degradative proteases in the lysosomes, but is larger than, and may be a precursor of, the lysosomal enzyme.

In addition, Bryan Noe and his colleagues at Emory University School of Medicine in Atlanta have isolated from the pancreatic islet cells of the anglerfish two distinct enzymes that perform different physiological cleavages of prosomatostatin. Finally, Paul Cohen and his colleagues at the Universite Pierre et Marie Curie in Paris, France, have also identified two possible prohormone-cleaving enzymes. One cleaves pro-oxytocin and the other works on prosomatostatin. The relation between Cohen's prosomatostatin-cleaving enzyme and those under study in Noe's laboratory is currently unclear.

Although the role of the *kex2* enzyme in cleaving the prohormone for the yeast mating factor has been firmly established, the status of the other proteases needs clarif tion. A recent gene transfer experiment p formed by the Thorner group in collab tion with Gary Thomas and Edward H bert of the University of Oregon He Sciences Center in Portland illustrates approach that will be used in the futur pin down the role of the mammalian cl age enzyme candidates.

When Thomas and Herbert transferr mouse POMC gene into mammalian c that do not ordinarily make POMC p tides, the prohormone was synthesized the cells, but there was no indication th was cleaved. A different result occu when the POMC gene was transferred gether with the yeast *kex2* gene, which been cloned by Anthony Brake and Lin Blair of the Berkeley group. Then, Tho says, "We got accurate cleavages and auth tic POMC peptides."

The yeast enzyme, which had never be encountered POMC, was nonetheless c ble of cleaving the mammalian prohorm at the correct locations. Thorner has s gested, and Steiner among others agr that the *kex2* enzyme may provide a m for the mammalian cleavage enzymes. ability of the yeast enzyme to work in m cells is consistent with this view, but d not constitute definitive proof for it.

Thorner says, however, that the *kex2* zyme does not resemble any of the o known proteases, including the propo mammalian cleavage enzymes. If the y enzyme is a prototype for the mamma cleavage enzymes, then additional prote that cut prohormones in higher animals still be found—and may supersede som all of the current candidates.

The purification of proteases that mi plausibly be mammalian cleavage enzy has opened the door to the eventual clon of the corresponding genes. Partial am acid sequences can now be determined probes constructed for identifying genes. Then transfer experiments simila those performed by Thomas and Her can be used to test the activities of enzymes in mammalian cells. In additi "anti-sense" messenger RNAs might be u to block the synthesis of a putative cleav enzyme and thus establish whether i necessary for the synthesis of one or m peptide hormones.

Most investigators think that eventual family of enzymes will prove to be neede cleave the large number of prohorm known to exist. How many of the prote described here will make the list remain be seen. As Noe says, "It's just too earl say [which are physiologically importa The burden of proof is with the in tigator." ■ JEAN L. MARX